Indonesian J. Pharm. Vol. 26 No. 1 : 52 – 56
ISSN-p : 2338-9427
DOI: 10.14499/indonesianjpharm26iss1pp56

Research Article

MACARANGIN, A GERANYLATED FLAVONOID AND ANTICANCER ACTIVE COMPOUND ISOLATED FROM ETHYL ACETAT FRACTION OF *Macaranga gigantifolia* LEAVES

Akhmad Darmawan[1,2,*], Wahyudi P. Suwarso[1], Soleh Kosela[1], Leonardus B.S. Kardono[2], Sofa Fajriah[2]

[1.] Dept. of Chemistry University of Indonesia Kampus Depok, Jl. Margonda Raya, Depok, West Java, Indonesia
[2.] Res. Center for Chemistry, Indonesian Institute of Scie., Kawasan PUSPIPTEK Serpong, Tangerang Selatan, Banten, Indonesia

Submitted: 12-10-2014
Revised: 10-11-2014
Accepted: 20-12-2014

*Corresponding author
Akhmad Darmawan

Email:
ahmaddarmawan@yahoo.com

ABSTRACT

Macaranga known locally as mahang-mahangan has uniquely ecological function, and also became a part of traditional medicine in Indonesia. *Macaranga* genus also known as a sources of terpenoid and phenolic (flavonoid) compounds which have biological activity as antioxidant and anticancer (cytotoxicity).There are few phytochemical investigations have been done on *M. gigantifolia* species. As a part of our continuing research of isolation anticancer compound from natural product, a geranylated flavonoid compound (macarangin) has been isolated from ethyl acetate fraction of *Macaranga gigantifolia* leaves using chromatography methods. The isolated compund (isolat MG) was elucidated to gain the chemical structure based on spectroscopic data (LC-MS and FT-NMR). Cytotoxicity test of this compound was tested against MCF 7 cell lines, showed that macarangin has a potential activity with IC_{50} value 119.12µg/mL.

Key words: Macarangin, geranylated flavonoid, Macaranga gigantifolia, MCF 7 cell lines.

INTRODUCTION

Macaranga (Euphorbiaceae), known locally as mahang-mahangan has uniquely ecological function, and also became a part of traditional medicine in Indonesia such as for diarrhea, wound and cough (Heyne, 1987). There are more than 308 species of *Macaranga,* wide-spread from Africa and west region of Madagascar until tropical region of Asia, North Australia and east region of Pacific Islands (Blattner, 2001). *Macaranga* genus also known as a sources of terpenoid (Hui, 1971)] and phenolic (flavonoid) (Jang, 2002; Schutz, 1995; Sutthivaiyakit, 2002; Yoder, 2007) compounds which have biological activity as antioxidant (Phommart, 2005) and anticancer (cytotoxicity) (Yoder, 2007). Macarangin, a geranylated flavonoid compound is one of the flavonoid typical compounds contained in *Macaranga* genus, and have been isolated from *M. vedeliana* (Hnawia, 1990) and *M. denticulate* (Sutthivaiyakit, 2002). Scopoletin is one of the secondary metabolite compounds was isolated from *M. gigantifolia* (Darmawan, 2012). There are few phytochemical investigations have been done on *M. gigantifolia* species. This study reports about the isolation and structure elucidation of macarangin from the ethyl acetate fraction of methanolic extract of *M. gigantifolia* leaves. This is the first reports about macarangin compound isolated from *M. gigantifolia.*

MATERIAL AND METHODS

M. gigantifolia leaves collected from Mekongga forest region, Kolaka District, Southeast Sulawesi - Indonesia in March 2013 and identified by Mr. Ismail Rachman from Herbarium Bogoriense, Research Center for Biology-LIPI.

Instrumentation

NMR spectra were recorded on a JEOL JNM-ECA 500 Spectrometer with TMS as an internal standard, and ESI-MS was performed on Mariner Shimadzu ESI system.

Procedure

Extraction and isolation

Two hundred milligram ethyl acetate fraction obtained from methanolic extract of *M. gigantifolia* leaves was dissolved in acetone, and chromatographed with chromatotron (centrifugal thin-layer chromatograph) using

gradient system solvent *n*-hexane – ethyl acetate (8:2 to 6:4) successively to obtain 12 fractions (F1-F12). F12 further separated by chromatotron using *n*-hexane-ethyl acetate (6:4) to afford 8 sub-fractions (FS-1 to FS-8) based on the TLC (Silica Gel GF 254, Merck) data. FS-6 further purified by preparative thin layer chromatography (TLC-P) to obtain **Isolate MG**.

Chemical compound characterization

Isolate MG characterized based on spectroscopic data obtained from FT-NMR and LC-MS analysis. FT-NMR and LC-MS analysis performed with JEOL JNM-ECA 500 MHz and Mariner Shimadzu ESI system.

Cytotoxicity test in vitro with Methyl Thyazole Tetrazolium (MTT) Assay

Isolate MG was tested *in vitro* for its cytotoxicity against MCF-7 cell line. MCF-7 cells growth inhibition by **Isolat MG** was analyzed 3-(4,5-dimethylthyazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay reported by Jin (2010) and Yunianto (2012). MCF-7 cells were seeded in 96 well plates and incubated with MTT (5mg/mL) for 4h. Cells further solubilized by added 100μL of DMSO. Absorbance was read at 570nm. Cell viability in treated cells was expressed as the amount of dye reduction relative compared to untreated control cells. The wells which contained only medium and 10mL of MTT were used as blanks for the plate reader.

RESULTS AND DISCUSSION

Chemical compound characterization

Isolate MG, a yellowish brown paste obtained from two step chromatography process using chromatotron with *n*-hexane:ethyl acetate as solvent system, gave a molecular ion [M+H] m/z 423.20 in ESI-MS, which mean that [M$^+$] is m/z 422.20.

FT-NMR: **Isolate MG**, a yellowish brown paste. ^{1}H-NMR (in acetone-d6, 500 MHz) (δ ppm, ΣH, *J* Hz) 1.54 (s, 3H, H-9"), 1.59 (s, 3H, H-10"), 1.79 (s, 3H, H-8"), 1.96 (dd, 1H, 8.4, H-4"), 2.07 (dd, 2H, 8.4/7.1, H-5"), 3.37 (d, 2H, 7.1, H-1"), 5.07 (t, 1H, 7.1, H-6"), 5.30 (t, 1H, 7.1, H-2"), 6.62 (s, 2H, H-8), 7.01 (d, 2H, 9.05, H-3'/H-5'), 8.13 (d, 2H, 8.4, H-2'/H-6'), 12.42 (s, 1H, -OH). ^{13}C-NMR (in acetone-d6, 125 Hz) (δ ppm) 16.32 (C-8"), 17.73 (C-9"), 25.87 (C-10"), 21.99 (C-1"), 27.44 (C-5"), 40.57 (C-4"), 93.97 (C-8), 103.97 (C-10), 111.86 (C-6), 116.36 (C-3'/5'), 123.22 (C-2"), 123.48 (C-1'), 125.19 (C-6"), 130.43 (C-2'/6'), 131.65 (C-7"), 135.37 (C-3"), 136.62 (C-3), 146.69 (C-2), 155.73 (C-9), 158.97 (C-5), 160.15 (C-4'), 163.10 (C-7), 176.58 (C-4, -C=O).

Based on the ^{1}H-NMR data showed that **Isolate MG** has 5 aromatic proton at δ 6.62, 7.01 (2H) and 8.13 (2H), 2 methine triplet signals at δ 5.07 and 5.30 typical for prenyl group, 1 hydroxyl group at δ 12.42, 3 methyl singlet at δ 16.26, 17.85 and 25.75, 3 methylene signals at δ 1.97, 2.07, and 3.37. ^{13}C-NMR data showed 25 carbon atoms consist of 5 aromatic methine signal at δ 93.9, 116.4 (2C), and 130.4 (2C), 11 quaternary carbon atoms at δ 103.97, 111.86, 123.48, 131.65, 135.37, 136.62, 146.69, 155.73, 158.97, 160.15, and 163.1, 1 carbonyl atom at δ 176.58. Proton signal at δ 7.01 (2H) and 8.13 (2H) which have correlation in ortho position indicates that this 4 proton located in the same aromatic ring. Proton aromatic (δ 6.62) indicates that there is another aromatic ring. 2 typical prenyl group methine correlated with only 3 methyl signals and 3 methyene signals indicates that the constituent is geranyl.

From the NMR data (^{1}H- and ^{13}C-NMR) indicates that **Isolat MG** is a geranylated flavonoid compound. Data HMBC-NMR showed that methylene group at δ 3.37 (H-1") correlated with three ring A carbon atom C-5, C-6, and C-7, it means geranyl substituent attached to the flavonoid aglycone through A ring (Figure 1.).

Based on NMR data, supported by LC-MS data and reference comparative (Table I.), **Isolat MG** was identified as macarangin (Figure 2) (Hnawia, 1990). This is the first report about macarangin content in *Macaranga gigantifolia.*

Cytotoxicity test in vitro with Methyl Thyazole Tetrazolium (MTT) Assay.

Cytotoxicity test (Table II) of **Isolat MG** was carried out at 100 ppm concentration using MTT viability test against MCF-7 cells (breast cancer cell) showed that **Isolat MG** could inhibit the growth of MCF-7 cell with IC_{50} value 119.12μg/mL (Figure 3). It means that **Isolat MG** has medium potential activity as breast cancer inhibitor.

Table I. ^{1}H- and ^{13}C-NMR data of **Isolate MG** and Macarangin

No.	Isolat MG		Macarangin (Hnawia, 1990)	
	δ_H (mult., ΣH, *J* in Hz)	δ_C	δ_H (mult., ΣH, *J* in Hz)	δ_C
2		146.69		146.00
3		136.62		135.61
4		176.58		177.25
5		158.97		160.35
6		111.86		112.32
7		163.10		163.10
8	6.62 (s, 1H)	93.97	6.43 (s, 1H)	93.70
9		155.73		156.13
10		103.97		104.28
1’		123.48		123.90
2’/6’	8.13 (d, 2H, 8.4)	130.43	8.10 (d, 2H, 8)	130.55
3’/5’	7.01 (d, 2H, 9.0)	116.36	6.90 (d, 2H, 8)	116.26
4’		160.15		159.04
1”	3.37 (d, 2H 7.15)	21.99	3.30 (d, 2H, 7)	22.11
2”	5.30 (t, 1H, 7.15)	123.22	5.25 (t, 1H, 7)	123.64
3”		135.37		136.97
4”	1.79 (s, 3H)	16.32	1.80 (s, 3H)	16.26
5”	1.97 (dd, 2H, 8.4)	40.57	2.00	40.64
6”	2.97 (dd, 2H, 8.4/7.15)	27.44	3.00	27.72
7”	5.07 (t, 1H, 7.15)	125.19	5.08 (t, 1H, 7)	125.46
8”		131.65		131.95
9”	1.54 (s, 3H)	17.73	1.57 (s, 3H)	17.85
10”	1.59 (s, 3H)	25.87	1.63 (s, 3H)	25.75

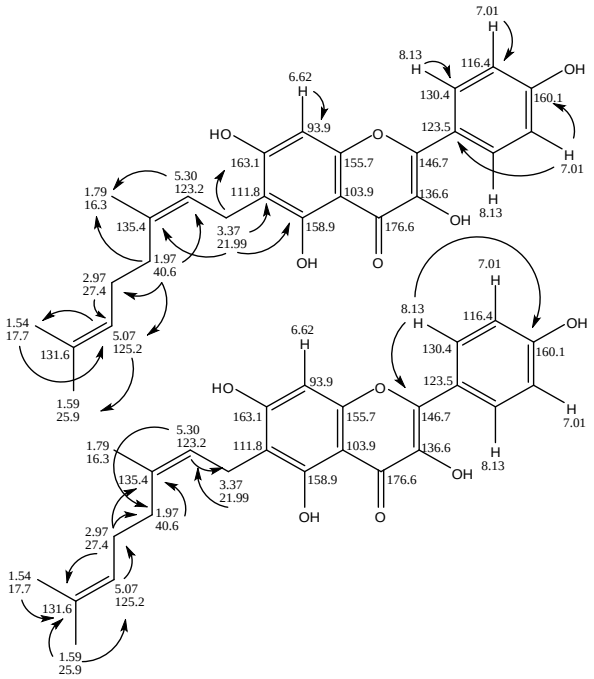

Figure 1. HMQC- and HMBC-NMR correlation of **Isolate MG** compound

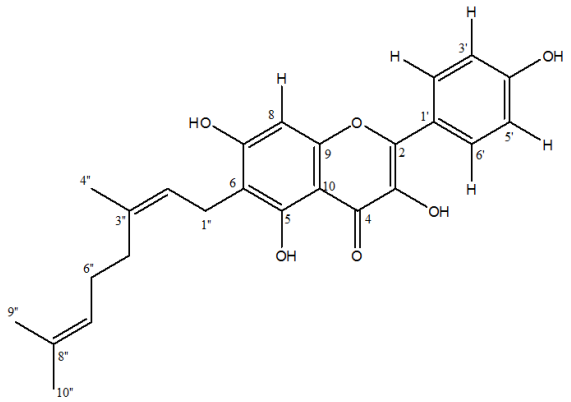

Figure 2. Chemical structure of macarangin

Table II. Anticancer activity of macarangin against MCF-7 cell lines

Concentration (µg/mL)	% Proliferation Inhibition
6.25	1.192053
12.5	3.046358
25	4.172185
50	7.284768
100	32.51656
200	95.49669

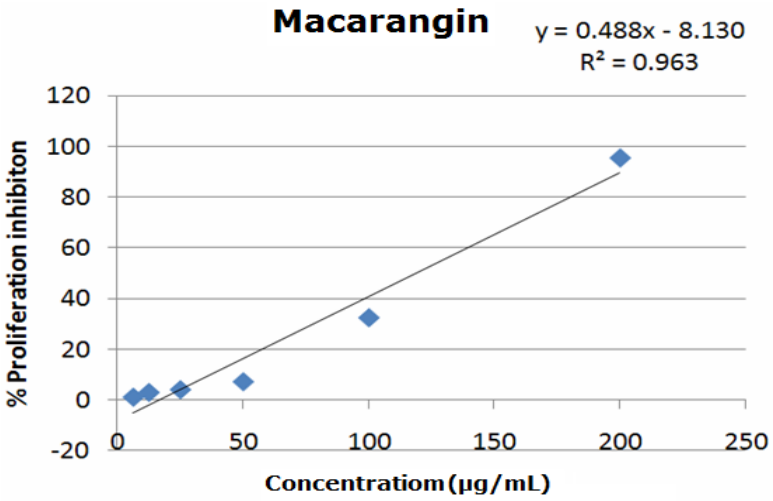

Figure 3. Anticancer activity of macarangin against MCF-7 cell lines

CONCLUSION

A geranylated flavonoid compound, macarangin (**Isolat MG**) has been isolated from the ethyl acetate fraction of *Macaranga gigantifolia* leaves. The cytotoxicity assay against MCF-7 cell lines showed that Macarangin has potential activity as breast cancer inhibitor with IC_{50} value 119.12µg/mL.

ACKNOWLEDGEMENTS

We thank to Prof. Dr. M. Hanafi and Mrs. Puspa Dewi, M.Eng. for chemical structure elucidation support.

REFERENCES

Blattner FR., Weising K., Banter G., Maschwitz U., Fiala B. 2001. Molecular Analysis of Phylogenetic Relationships among Myrmecophytic *Macaranga* Species (Euphorbiaceae). Molecular Phylogenetics and Evolution. 19(3):331-344.

Darmawan A., Kosela S., Kardono LBS., Syah YM. 2012. Scopoletin, a coumarine derivative compound isolated from *Macaranga gigantifolia* Merr. Journal of Applied Pharmaceutical Science. 2(12):175-177.

Heyne K. 1987. *Tumbuhan Berguna Indonesia,* Jilid I. Yayasan Sarana Wanajaya, Jakarta. 1163-1165.

Hnawia E., Thoison O., Voegelein FG., Bourret D., Sevenet T. 1990. A Geranyl Substituted Flavonol from *Macaranga vedeliana*. Phytochemistry. 29:2367-2368.

Hui WH., Ng KK. 1971. Isolation and Structure of Macarangonol, A Diterpene Ketol from *Macaranga tanarius*. Phytochemistry. 10:1617-1620.

Jang DS., Cuendet M., Hawthorne ME., Kardono LBS., Kawanishi K., Fong, HHS., Mehta RG., Pezzuto JM., Kinghorn AD. 2002. Prenylated Flavonoids of The Leaves of *Macaranga conifera* with Inhibitory Activity against Cyclooxygenase-2. Phytochemistry. 61:867-873.

Jin S., Zhang QY., Kang XM., Wang JX., Zhao WH. 2010. Daidzein induced MCF-7 breast cancer cell apoptosis via the mitochondrial pathway. Annals of Oncology. 21:263-268.

Phommart S., Sutthivaiyakit P., Chimnoi N., Ruchirawat S., Sutthivaiyakit S. 2005. Constituents of the Leaves of *Macaranga tanarius*. *J Nat. Prod.* 6:927-930.

Schutz BA., Wright AD., Rali T., Sticher O. 1995. Prenylated Flavanones from Leaves of *Macaranga pleiostemona*. Phytochemistry. 40:1273-1277.

Sutthivaiyakit S., Unganont S., Sutthivaiyakit P., Suksamrarn A. 2002. Diterpenylated and Prenylated Flavonoids from *Macaranga denticulata*. Tetrahedron. 58:3619-3622.

Yoder BJ., Cao S., Norris A., Miller JS., Ratovoson F., Razafitsalama J., Andriantsiferana R., Rasamison VE., Kingston GI. 2007. Antiproliferative Prenylated Stilbenes and Flavonoids from *Macaranga alnifolia* from the Madagascar Rainforest. Journal of Natural Products. 70:342-346.

Yunianto P., Rosmalawati S., Rachmawati I., Suwarso WP., Sumaryono, W. 2012. Isolation and Identification of Endophytoc Fungi from Srikaya Plants (*Annona squamosa*) Having Potential Secondary Metabolites as Anti-Breast Cancer Activity. Microbiology Indonesia. 6(1):23-29.